



0327-0759-0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF:

:

NAOKO TSUJI ET AL.

: GROUP ART UNIT: 1651

SERIAL NO: 09/220,691

:

FILED: DECEMBER 28, 1998

: EXAMINER: WEBER

FOR: METHOD OF INHIBITING HAIR GROWTH

DECLARATION UNDER 37 C.F.R. 1.132HONORABLE COMMISSIONER OF PATENTS & TRADEMARKS
WASHINGTON, D.C. 20231

SIR:

Now comes Naoko Tsuji, who deposes and states that:

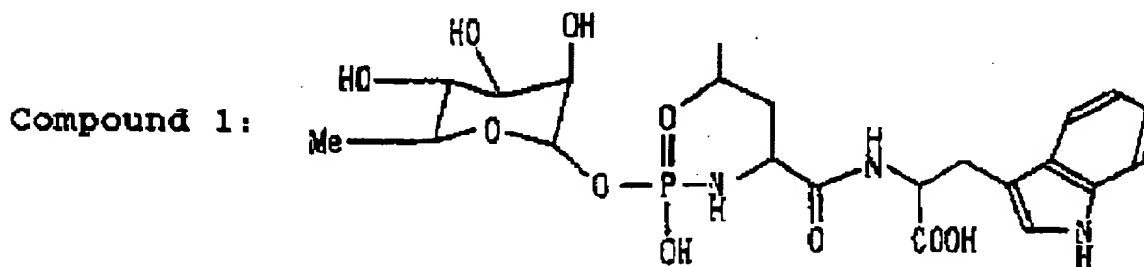
1. I am a graduate of Tokyo University of Agriculture & Technology, Faculty of Agriculture, Department of Agricultural Chemistry and received my Master degree in the year 1992.

2. I have been employed by Kao Corporation of Japan as a researcher in the Kao Biological Science Laboratories or the Kao Institute for Fundamental Research for a total of 9.5 years.

3. The following experiments were carried out by me or under my direct supervision and control, and are provided to show the measurement of Type I collagenase (MMP-1) activity for Compound 1 at page 14 of the present application.

Example:

Type I collagenase activity was measured using a conventional and commercially available assay kit obtained from YAGAI Corp (Yamagata, Japan). The method used was as follows: Type I collagen was labeled by fluorescein isothiocyanate (FITC). 0.1 unit/ml of Type I collagenase, from human skin fibroblasts, and Compound 1 (shown below):



were incubated in 0.2 M NaCl, 5 mM CaCl₂, and 0.05 M Tris HCl (pH 7.5) buffer. FITC-labeled substrate was added and incubated for 2 h at 37°C. The unreacted substrate was precipitated by ethanol and removed by centrifugation at 8,000 rpm for 10 min. The release of denatured collagen was measured for fluorescence intensity in the supernatant at 495 nm and 520 nm for excitation and emission, respectively.

Compound 1 was found to have inhibitory activities on Type I collagenase of 0% at both of 1 mM and 0.1 mM concentrations. This indicates that Compound 1 has no inhibitory activity on Type I collagenase (MMP-1).

4. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.